PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIOXIDANT ACTIVITY OF MEMECYLON UMBELLATUM ROOT EXTRACTS

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ABSTRACT
The objective of this study to find out the DPPH scavenging (antioxidant) activity of dry extract of Memecylon umbellatum root obtained by various solvents such as petroleum ether, chloroform, ethyl acetate, acetone, methanol and chloroform water (IP). The in-vitro free radical scavenging activity was recorded by DPPH method using various concentrations of dry extract in distilled water (1, 2, 4, 8, 16, 20µg/ml) against blank with ascorbic acid as a standard in same concentrations. From among the extract the acetone extract showed maximum 95.770±0.042% antioxidant activity at 20µg/ml while petroleum ether shows minimum 5.290±0.0482%. The IC₅₀ value of acetone extract was found to be 10.44±0.0846µg/ml as compare to standard ascorbic acid 3.76±0.0856µg/ml. By using Graphpad Instat 3 software paired t test of acetone root extract exhibits significant results (p<0.05) compared to standard ascorbic acid. In future it may found as a main source for dietary antioxidants and nutrient.

KEYWORDS: Memecylon umbellatum, DPPH reagent, Antioxidant activity, IC₅₀.

INTRODUCTION
Now-a-days world moving faster and every technology and service came in a nutshell of single word ‘globalization’ but this fast moving world brings very unhealthy and unhygienic lifestyle of fast food, 10-12 working hours, a diet which is lack of nutrients. Ultimately this all results in the increased oxidative stress in normal routine life of a human being. The oxidative stress causes various physiological and psychological disorders some common examples are atherosclerosis, heart disease, ageing, diabetes mellitus, immunosuppression, nervous disorders and others. [1, 2] To regulate the disorder the helping hand came out in the
form of synthetic antioxidant and various food supplements containing antioxidant, but these synthetic antioxidant capsules and dietary supplements are found to be less effective in various cases. In response to satisfy the thrust of antioxidants many more medicinal plants were found which containing natural antioxidants has shown beneficial therapeutic potentials including *Osmium sanctum*, *Allium sativum* Linn, *Terminalia bellerica*, *Zingiber officinale Roscoe* and several Indian plants. The majority of the antioxidant activity is due to the flavones, is flavones, flavonoids, anthocyanin, catechins and isocatechins.\[^{[3]}\]

*Memecylon umbellatum* Burm. (Family: Melastomataceae) is a small evergreen shrub or tree having young tree branches and bears numerous umbellate cymes. The plant is known as “Anjani” in Sanskrit and “Ironwood tree” in English. Plants are distributed mostly in coastal regions of the Deccan peninsula, the eastern and southern part of India all along the Western Ghats and in the Andaman islands.\[^{[4, 5]}\] The leaves have been reported to possess astringent properties and are given to treat leucorrhoea and gonorrhea. Lotion prepared from leaves is used to treat eyetroubles. The decoction of the root is used in the treatment of excessive menstrual discharge.\[^{[6]}\] Leaves are also reported to possess antiviral activity.\[^{[7]}\] Bark is used in the treatment of bruises externally as leap along with coconut kernels (Dymock). The literature survey reveals that the leaves and roots of *Memecylon umbellatum* have been investigated for its hypoglycemic activity using alloxan induced hyperglycemia Wistar albino rats.\[^{[8, 9]}\] Wound healing activity of ethanolic extract of the leaves has also been reported.\[^{[10]}\] Plant contains a wide variety of phytoconstituents such as umbellactone, β-amyrine, oleanolic acid, ursolic acid, sitosterol and organic acids.\[^{[11, 12]}\]

**MATERIAL AND METHODS**

The roots of *Memecylon umbellatum* were collected in the month of March-April from Gaganbawda region, Maharashtra, India. The plant material was taxonomically identified by Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.). The voucherspecimen is deposited in the Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur. A standard curve was obtained using Ascorbic acid (ACME Chemicals, Mumbai) with the help of double beam UV/Visible spectrophotometer (Jasco-V-630). All other solvent and chemicals were of AR grade and procured from LobaChem.

**Preparation of extract**

Roots were washed thoroughly in running water and dried under shade for 15-20 days. Roots
were powdered using heavy duty Willey type Disintegrator (S. G. Phytopharma, Gokul-Shirgaon, Kolhapur) fine powder of the root (# 180) was used for extraction.

**Soxhlet extraction process**

One kg powder of roots of *Memecylon umbellatum* was used for extraction. Sample powder was packed gently in previously washed and dried cloth bag and solvent was placed from the top with the help of funnel to moisten the drug sample. 3.5 liter of solvent (ethyl acetate, methanol, chloroform water, chloroform, and petroleum ether) was placed in distillation flask and assembly was made air tight with sealing wax. Solvents were selected on the basis of extractive values and with their increasing order of polarity. Extraction was carried out at or slightly above the boiling point of each solvent. Extraction was carried out for 18 hours or on the basis of clarity of dropping solvent (saturation). The solvent was collected every time after completion of the process and powder was dried in hot air oven for 24h at 45°C. The process was repeated for all the next solvents and finally the dried powder was macerated with 3.5 liter of chloroform water IP (0.25% v/v) at room temperature with frequent shaking. All the liquid extracts were subjected for physical analysis and are concentrated in a rotary film vacuum evaporator (Dolphin, Mumbai) and finally dried under reduced pressure. The residue was weighed and % yield was calculated (table number.1). All the extracts were further dried over anhydrous calcium chloride and preserved in vacuum desiccators for further studies. Different extracts were abbreviated according to solvent and part of the plant and used throughout the work. The assembly process of soxhletion was carried out as per the figure no. 1

**Table No. 1: Percent yield of *Memecylon umbellatum* root extract**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Solvents used for extraction</th>
<th>Yield per Kilogram of crude drug in grams</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet. Ether</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>0.6</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate</td>
<td>1.03</td>
<td>0.103</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>14.25</td>
<td>1.425</td>
</tr>
<tr>
<td>5</td>
<td>Methanol</td>
<td>12.60</td>
<td>1.26</td>
</tr>
<tr>
<td>6</td>
<td>Chloroform water</td>
<td>9.72</td>
<td>0.972</td>
</tr>
</tbody>
</table>

**Reagents for antioxidant activity**

1. DPPH Reagent: Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved in one liter of analytical grade methanol.
2. Standard solution: Ascorbic acid was used as standard in following concentrations 1, 2, 4, 8, 16, 20 µg/ml in methanol.

3. Sample preparation: Test samples of each dry extract were prepared by dissolving in distilled water in the various concentrations as 1, 2, 4, 8, 16, 20µg/ml.

Experimental Methods

Phytochemical screening

The phytochemical screening was performed by following method, about 500 mg of each dried extract was dissolved in 100 ml of respective solvent and the solution obtained was subjected to phytochemical screening using different specific and general reagents. Samples were prepared as per the requirement of procedure and tests were repeated for final confirmation of phytoconstituents. Different extracts showing active constituents present or absent have shown separately for individual parts of plant studied in table number 2.

Table No. 2: Phytochemical screening of root extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Sugars</th>
<th>Alk.</th>
<th>Tannins</th>
<th>Glycosides</th>
<th>Steroids</th>
<th>Proteins</th>
<th>Org. acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R RNR</td>
<td>HT CT</td>
<td>a c s f</td>
<td>co ST TT</td>
<td>C O T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AqER</td>
<td>+ + - +</td>
<td>- -</td>
<td>+ - - +</td>
<td>- - - -</td>
<td>- + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MER</td>
<td>+ + - +</td>
<td>+ +</td>
<td>+ - + +</td>
<td>+ - + -</td>
<td>- + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AER</td>
<td>+ + - +</td>
<td>- +</td>
<td>- + + +</td>
<td>+ - + -</td>
<td>- + -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAER</td>
<td>+ + - +</td>
<td>- -</td>
<td>- + - -</td>
<td>- - - +</td>
<td>- - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChER</td>
<td>+ + - -</td>
<td>- -</td>
<td>- - - -</td>
<td>+ - - -</td>
<td>- - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEER</td>
<td>- + - -</td>
<td>- -</td>
<td>- - - -</td>
<td>+ - - -</td>
<td>- - -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Screening of extracts for in- vitro antioxidant activity using DPPH Assay: [13-17]

Principle:
The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:
Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence there is decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

**Procedure**

The DPPH scavenging activity was performed using a solution of 0.1 mM DPPH in methanol solution and 1.0 ml solution was added in 3.0 ml of test samples of each dry extract having concentrations as 1, 2, 4, 8, 16 and 20µg/ml in methanol and kept in darkness. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding the extract. Ascorbic acid at concentration 1, 2, 4, 8, 16, 20µg/ml was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

\[
\text{DPPH Scavenged (\%) } = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100
\]

Where ‘A control’ is the absorbance of the control reaction and ‘A test’ is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the different extract was expressed in % DPPH radical scavenged and the results are given in table number 3.

**IC\textsubscript{50}** value was determined to express antioxidant activity. It is the concentration of fractions that inhibits the formation of DPPH radicals by 50%.

**Table No. 3: Observations for DPPH scavenging activity**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>% Activity of extract with various concentrations in (µg/ml)</th>
<th>IC\textsubscript{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>Standard</td>
<td>25.13±0.0262</td>
<td>39.68±0.0161</td>
</tr>
<tr>
<td>2</td>
<td>Petroleum ether</td>
<td>0.2645±0.0196</td>
<td>0.529±0.0145</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>0.3965±0.0842</td>
<td>0.7930±0.0874</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl</td>
<td>3.571±0.09</td>
<td>7.142±0.14284</td>
</tr>
</tbody>
</table>
### RESULTS AND DISCUSSION

**Phytochemical screening of different extracts**

In the phytochemical screening of extracts shows the presence of reducing and nonreducing in almost every extract. In the further screening it was found that alkaloids are absent in every extract. The hydrolyzable tannins were found in the aqueous, methanol, acetone and ethyl acetate extract but condensed tannins found only in methanol and ethyl acetate extract. Some glycosides were traced like cardiac, saponin, flavanoidal and coumarin glycosides in the methanol and acetone extract. Steroids and triterpenes were also found to be present in acetone, ethyl acetate, chloroform and petroleum extract. Some extracts shows presence of proteins and organic acids also. As extracts showing presence of triterpenes and polyphenolic compounds the further investigation was carried out with antioxidant activity.

**Screening of different extracts for In-vitro antioxidant activity**

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing oxidative stress. These herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated the free radical scavenging activity of various methanolic root extracts of Memecylon umbellatum. Antioxidant activity was tested by using DPPH (2, 2 Di Phenyl 2 PicrylHydrazyl) reagent. Acetone extract of root shown better antioxidant activity compares to control and other extracts tested. The Acetone root extract has shown 95.77% of antioxidant activity at 20µg/ml as compare to other (Ethyl acetate, Methanol, Aqueous, Chloroform, Petroleum ether i.e. 71.42%, 69.84%, 46.56%, 7.93%, 5.29% respectively) root extracts. Chloroform, Petroleum ether root extracts shows very weak or negligible activity. All the antioxidant activities in different concentrations are shown as a graph in figure 1. From the antioxidant activity the IC$_{50}$ values of test and standard samples were determined. The standard i.e. ascorbic acid shows 3.76 IC$_{50}$ value. In the various extracts the acetone root shows 10.44

<table>
<thead>
<tr>
<th></th>
<th>acetate</th>
<th>Acetone*</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Petroleum ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.0451</td>
<td>0.0955</td>
<td>0.0215</td>
<td>0.0585</td>
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<td>6</td>
<td>0.0485</td>
<td>0.155</td>
<td>0.155</td>
<td>0.0564</td>
<td>0.036</td>
</tr>
<tr>
<td>7</td>
<td>0.065</td>
<td>0.0898</td>
<td>0.0658</td>
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<tr>
<td></td>
<td>0.0626</td>
<td>0.0898</td>
<td>0.0658</td>
<td>0.0644</td>
<td>0.036</td>
</tr>
</tbody>
</table>

*indicates mean value of triplicate readings of ± Standard deviation

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IC₅₀ values which is less in all extracts. After the acetone IC₅₀ value ethyl acetate, methanol, and aqueous extract shows significant IC₅₀ value which was 14, 14.21, and 21.47. The other extracts of root Chloroform and Pet. Ether shows very high IC₅₀ value which was 126.10 & 189.03. The experimental analysis of all extracts showed that for all the examined root extracts rank order in terms of % antioxidant activity efficiency was always: Acetone > Ethyl acetate > Methanol > Aqueous > Chloroform > Pet. Ether. But it was also observed that all the sample extracts have lesser activity than that of standard ascorbic acid which was found IC₅₀ value 3.76 in the methanolic solution. The % Antioxidant activity of *Memecylon umbellatum* root extract with different solvents at 20 µg/ml concentration in distilled water were shown as chart in figure 2.

**Fig.1:** Plot of % DPPH radical scavenging activity versus concentration of standard and different fractions.

**Fig.2:** IC₅₀ values of *Memecylon umbellatum* root extract with different solvents at 20 µg/ml concentration in distilled water.
CONCLUSION
This investigation reveals the effect of different extracts of *Memecylon umbellatum* root on antioxidant activity. In this experimental study, it was found that acetone extract shows significant (p<0.05) antioxidant activity having IC\textsubscript{50} value 10.44±0.0846 µg/ml as compare to standard ascorbic acid. In future this plant may found the main source for dietary supplement as an antioxidant so there is further need of study in this area to find out the active constituents responsible for antioxidant activity.

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